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USPT,JPAB,EPAB,DWPI	fil	5457	<u>L4</u>
USPT,JPAB,EPAB,DWPI	l1 near15 l2	35	<u>L3</u>
USPT,JPAB,EPAB,DWPI	stress near10 (resist\$8 or toler\$7)	43109	<u>L2</u>
USPT,JPAB,EPAB,DWPI	yeast\$1	66408	<u>L1</u>

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Jan 12, 1994

DERWENT-ACC-NO: 1994-009855

DERWENT-WEEK: 199402

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TITLE: Transformed yeast with increased stress resistance or fermentation capacity
- has modification in general glucose sensor system, partic. for bread-making, but
also prodn. of alcohol or foreign proteins

INVENTOR: HOHMANN, S; THEVELEIN, J ; VAN DIJCK, P

PRIORITY-DATA: 1992EP-0870102 (July 9, 1992)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
EP 577915 A1	January 12, 1994	F	026	C12N015/81

INT-CL (IPC): A21D 8/04; C07K 15/00; C12N 1/18; C12N 9/12; C12N 15/81

ABSTRACTED-PUB-NO: EP 577915A

BASIC-ABSTRACT:

New yeast strain is transformed so that it has resistance to stress and/or altered sugar metabolism (partic. increased fermentation capacity). It has a modification in at least one of the general glucose sensor systems consisting of at least (a) a protein serving as general glucose sensor and coded by GGS1 or a similar gene; (b) a glucose membrane transport protein of low affinity and (c) a sugar kinase.

The modifications of the gene(s) encoding these proteins (and/or of the promoters and 3'- flanking sequences) confer new properties to the transformed strain for its prodn. and/or use as industrial yeast.

Partic. the yeast has a modification in a GGS1 gene (or allele or related gene) or in promoter or flanking region. In partic. this gene can be placed under control of a constitutive promoter to render its expression at least partly independent of glucose/nitrogen regulation in the culture medium.

USE/ADVANTAGE - The new strains are esp. useful in breadmaking and have resistance to at least one of drying; osmotic shock (esp. in sugar-contg. dough) and freezing, and/or better survival in frozen doughs. Pref. it also has higher trehalose content with delayed trehalose mobilisation. Apart from use in breadmaking, the strains can also be used to produce alcohol or beverages, heterologous proteins and yeast biomass.

ABSTRACTED-PUB-NO: EP 577915A

EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/8

> d 113 7, 12, 14, 28, 38 bib hit

L13 ANSWER 7 OF 39 CAPLUS COPYRIGHT 2001 ACS

AN 1996:722433 CAPLUS

DN 126:30537

TI Leavening ability and **freeze tolerance** of
yeasts isolated from traditional corn and rye bread doughs

AU Almeida, M. J.; Pais, C.

CS Dep. Biol., Univ. Minho, Braga Codex, 4709, Port.

SO Appl. Environ. Microbiol. (1996), 62(12), 4401-4404
CODEN: AEMIDF; ISSN: 0099-2240

PB American Society for Microbiology

DT Journal

LA English

TI Leavening ability and **freeze tolerance** of
yeasts isolated from traditional corn and rye bread doughs

SO Appl. Environ. Microbiol. (1996), 62(12), 4401-4404

CODEN: AEMIDF; ISSN: 0099-2240

AB Strains of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* isolated from traditional bread doughs displayed dough-raising capacities similar to the ones found in baker's yeasts. During storage of frozen doughs, strains of *T. delbrueckii* (GC 5321, IGC 5323, and IGC 4478) presented approx. the same leavening ability for 30 days. Cell viability was not significantly affected by freezing, but when the dough was submitted to a bulk fermn. before being stored at -20.degree.C, there was a decrease in the survival ratio which depended on the yeast strain. Furthermore, the leavening ability after 4 days of storage decreased as the

prefermentation

period of the dough before freezing increased, except for strains IGC

5321

and IGC 5323. These two strains retained their **fermentative** activity after 15 days of storage and 2.5 h of prefermentation, despite showing a redn. of viable cells under the same conditions. The intracellular trehalose content was higher than 20% (wt/wt) in four of

the

yeasts tested: the two com. strains of baker's yeast (*S. cerevisiae* IGC 5325 and IGC 5326) and the two mentioned strains of *T. delbrueckii* (IGC 5321 and IGC 5323). However, the strains of *S. cerevisiae* were clearly more susceptible to **freezing** damages, indicating that other factors may contribute to the **freeze tolerance** of these **yeasts**.

ST leavening ability **freeze tolerance yeast**

dough; *Saccharomyces* leavening ability freeze tolerance dough;

Torulaspora

leavening ability freeze tolerance dough

IT Frozen foods

(frozen dough; leavening ability and **freeze tolerance**
of **yeasts** isolated from traditional corn and rye bread
doughs)

IT Dough

(frozen; leavening ability and **freeze tolerance** of
yeasts isolated from traditional corn and rye bread doughs)

IT Cold effects (biological)

Saccharomyces cerevisiae

Torulaspora delbrueckii

(leavening ability and **freeze tolerance** of
yeasts isolated from traditional corn and rye bread doughs)

IT 99-20-7, Trehalose 124-38-9, Carbon dioxide, biological studies

RL: MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)

(leavening ability and **freeze tolerance** of
yeasts isolated from traditional corn and rye bread doughs)

L13 ANSWER 12 OF 39 CAPLUS COPYRIGHT 2001 ACS
AN 1995:361606 CAPLUS
DN 122:128478
TI Breeding of **freeze-tolerant yeast** and the
mechanisms of **stress tolerance**
AU Hino, Akihiro
CS Natl. Food Res. Inst., Ministry Agriculture, Tsukuba, 305, Japan
SO Nippon Reito Kyokai Ronbunshu (1994), 11(3), 247-62
CODEN: NRKRET; ISSN: 0910-0040
DT Journal
LA Japanese
TI Breeding of **freeze-tolerant yeast** and the
mechanisms of **stress tolerance**
SO Nippon Reito Kyokai Ronbunshu (1994), 11(3), 247-62
CODEN: NRKRET; ISSN: 0910-0040
AB Frozen dough method have been adopted in the baking industry to reduce
labor and to produce fresh breads in stores. New **freeze-**
tolerant yeasts for frozen dough preps. were isolated
from banana peel and identified. To obtain strains that have
fermentative ability even after several mo. of frozen storage in
fermented dough, the authors attempted to breed new
freeze-tolerant strain. Freeze-tolerant strains showed higher surviving
and trehalose accumulating abilities than freeze-sensitive strains. The
freeze tolerance of the **yeasts** was assocd.
with the basal amt. of intracellular trehalose after rapid degrdn. at the
onset of the prefermn. period. The complicated metabolic pathway and the
regulation system of trehalose in yeast cells are introduced. The
trehalose synthesis may act as a metabolic buffer system which
contributes
to maintaining the intracellular inorg. phosphate and as a feedback
regulation system in the glycolysis. However, it is not known how the
trehalose protects yeast cells from stress.
IT **Stress**, biological
(cold, trehalose in relation to cold **tolerance** in
yeast)

L13 ANSWER 14 OF 39 CAPLUS COPYRIGHT 2001 ACS
AN 1995:244890 CAPLUS
DN 122:30180
TI Improvement of **freeze tolerance** of commercial bakers'
yeasts in dough by **heat** treatment before
freezing
AU Nakagawa, Satoshi; Ouchi, Kozo
CS Tokyo Research Laboratories, Kyowa Hakko Kogyo, Co., Ltd., Tokyo, 194,
Japan
SO Biosci., Biotechnol., Biochem. (1994), 58(11), 2077-9
CODEN: BBBIEJ; ISSN: 0916-8451
DT Journal
LA English
TI Improvement of **freeze tolerance** of commercial bakers'
yeasts in dough by **heat** treatment before
freezing
SO Biosci., Biotechnol., Biochem. (1994), 58(11), 2077-9
CODEN: BBBIEJ; ISSN: 0916-8451
AB Although fully **fermented** doughs with a non-freeze-
tolerant yeast lost **fermentative** activity
after frozen storage, **heat** treatment at 46.degree.C for 10 min
of the **fermented doughs** greatly improved the **freeze tolerance**.
The sp. vol. increased and the proof time decreased. The heat treatment
was effective for the straight method for white dough and also for the

sponge and dough methods for sweet dough.

ST dough **heat** bakers' **fast freeze**
tolerance

IT Dough
Freezing
(improvement of **freeze tolerance** of com. bakers'
yeasts in dough by **heat** treatment before
freezing)

IT **Yeast**
(bakers', improvement of **freeze tolerance** of com.
bakers' **yeasts** in dough by **heat** treatment before
freezing)

IT Frozen foods
(dough, improvement of **freeze tolerance** of com.
bakers' **yeasts** in dough by **heat** treatment before
freezing)

IT Dough
(frozen, improvement of **freeze tolerance** of com.
bakers' **yeasts** in dough by **heat** treatment before
freezing)

IT Temperature effects, biological
(**heat**, improvement of **freeze tolerance** of
com. bakers' **yeasts** in dough by **heat** treatment
before **freezing**)

L13 ANSWER 28 OF 39 CAPLUS COPYRIGHT 2001 ACS
AN 1989:189044 CAPLUS
DN 110:189044
TI The relationship between **freezing resistance** and fatty
acid composition of **yeasts**
AU Sajbidor, J.; Breierova, E.; Kockova-Kratochvilova, A.
CS Fac. Chem., Slovak Tech. Univ., Bratislava, 812 37, Czech.
SO FEMS Microbiol. Lett. (1989), 58(2-3), 195-8
CODEN: FMLED7; ISSN: 0378-1097
DT Journal
LA English
TI The relationship between **freezing resistance** and fatty
acid composition of **yeasts**
SO FEMS Microbiol. Lett. (1989), 58(2-3), 195-8
CODEN: FMLED7; ISSN: 0378-1097
AB The relationship between **freezing resistance** and
cellular long-chain fatty acid compn. of 18 selected **yeast**
strains were studied. All strains produced a series of satd. and unsatd.
even-numbered fatty acids ranging 14-20 carbons in length. The majority
of the **freeze-resistant yeasts** were found
among **fermentative** species with a content of oleic acid >40%.
ST **yeast fatty acid freezing resistance**
IT **Yeast**
(fatty acid compn. and **freezing resistance** in)
IT **Freezing**
(**resistance** to, in **yeast**, fatty acid compn. in
relation to)
IT Fatty acids, biological studies
RL: BIOL (Biological study)
(long-chain, of **yeast**, **freezing resistance**
in relation to)

L13 ANSWER 38 OF 39 MEDLINE
AN 97197175 MEDLINE
DN 97197175 PubMed ID: 9044264
TI Stationary-phase regulation of the *Saccharomyces cerevisiae* SOD2 gene is
dependent on additive effects of HAP2/3/4/5- and STRE-binding elements.
AU Flattery-O'Brien J A; Grant C M; Dawes I W
CS School of Biochemistry and Molecular Genetics, University of New South
Wales, Sydney, Australia.

SO MOLECULAR MICROBIOLOGY, (1997 Jan) 23 (2) 303-12.
Journal code: MOM; 8712028. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199705
ED Entered STN: 19970609
Last Updated on STN: 19970609
Entered Medline: 19970527

SO MOLECULAR MICROBIOLOGY, (1997 Jan) 23 (2) 303-12.
Journal code: MOM; 8712028. ISSN: 0950-382X.

AB SOD2, encoding manganese superoxide dismutase (MnSOD), is essential for stationary-phase survival of **yeast** cells. In addition, stationary-phase cells are more **resistant** to oxidative **stress** than exponential-phase cells. The use of a SOD2::lacZ fusion construct in this study shows that transcription of SOD2 increases 6.5-fold as cells enter stationary phase in rich, glucose medium. The increase in SOD2 expression appears to be due to two phenomena-the switch to a non-**fermentable** carbon source and nutrient limitation. Analysis of SOD2 transcription in mutant *Saccharomyces cerevisiae* strains showed that the gene was negatively regulated by intracellular cAMP

levels

which decrease as cells enter stationary phase. Mutation of 'stress-responsive' (STRE) elements in the SOD2 promoter which respond to cAMP levels resulted in the loss of cAMP-dependent expression but only partially reduced the increase in expression as cells entered stationary phase. A putative Yap1p-binding site was found to be inactive and

mutation

of YAP1 had no effect on the STRE-mediated expression. To fully eliminate the stationary-phase response, it was necessary to mutate a HAP2/3/4/5 complex binding site in addition to the STRE elements. It is postulated that the effects of the STRE sites and the HAP2/3/4/5 complex binding

site

are additive.

d 118 1-17 bib hit

L18 ANSWER 1 OF 17 CAPLUS COPYRIGHT 2001 ACS

AN 2000:813005 CAPLUS

TI Tolerance mechanism of the ethanol-tolerant mutant of sake yeast

AU Ogawa, Yoshiaki; Nitta, Asako; Uchiyama, Hirofumi; Imamura, Takeshi; Shimoi, Hitoshi; Ito, Kiyoshi

CS Tatsuumma-honke Brewing Co. Ltd., Nishinomiya, 662-0943, Japan

SO J. Biosci. Bioeng. (2000), 90(3), 313-320

CODEN: JBBIF6; ISSN: 1389-1723

PB Society for Bioscience and Bioengineering, Japan

DT Journal

LA English

RE.CNT 45

RE

(2) Bell, W; Eur J Biochem 1992, V209, P951 CAPLUS

(3) Boeke, J; Mol Gen Genet 1984, V197, P345 CAPLUS

(4) Boone, C; J Cell Biol 1990, V110, P1833 CAPLUS

(5) Bradford, M; Anal Biochem 1976, V72, P248 CAPLUS

(6) Bussey, H; J Bacteriol 1979, V140, P888 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

IT Gene, microbial

RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(GPD1, CTT1, CYC7, HSP12, HOR7, SPI1, TPS1, and TPS2; **tolerance**
mechanism of the ethanol-tolerant mutant of sake
yeast involves differential expression of **stress**
-responsive genes in the absence of ethanol and ethanol-induced mRNA
expression)

IT Saccharomyces cerevisiae

(SR4-3; **tolerance** mechanism of the ethanol-tolerant
mutant of sake **yeast** involves differential mRNA
expression of **stress**-responsive genes in the absence of
ethanol and ethanol-induced mRNA)

IT Transcriptional regulation

(activation; **tolerance** mechanism of the ethanol-
tolerant mutant of sake **yeast** involves
differential expression of **stress**-responsive genes in the
absence of ethanol and ethanol-induced mRNA expression)

IT Stress, microbial

(**heat**, osmotic, and oxidative; **tolerance** mechanism
of the ethanol-tolerant mutant of sake
yeast involves differential expression of **stress**
-responsive genes, accumulation of **stress** protective
substances, and multiple-stress resistance)

IT 9001-05-2, Catalase

RL: BAC (Biological activity or effector, except adverse); BIOL
(Biological study)
(**tolerance** mechanism of the ethanol-tolerant
mutant of sake **yeast** involves differential expression
of **stress**-responsive genes in the absence of ethanol,
accumulation of stress protective substances, and multiple-stress
resistance)

IT 56-81-5, Glycerol 99-20-7, Trehalose

RL: BOC (Biological occurrence); MFM (Metabolic formation); BIOL
(Biological study); FORM (Formation, nonpreparative); OCCU (Occurrence)
(**tolerance** mechanism of the ethanol-tolerant
mutant of sake **yeast** involves differential expression
of **stress**-responsive genes in the absence of ethanol,

L18 ANSWER 2 OF 17 CAPLUS COPYRIGHT 2001 ACS
AN 2000:327624 CAPLUS
TI Stress tolerance in yeast.
AU Watson, K.
CS Department of Biological Sciences, University of New England, Armidale, NSW, 2351, Australia
SO Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000), BTEC-005 Publisher: American Chemical Society, Washington, D. C.
CODEN: 69CLAC
DT Conference; Meeting Abstract
LA English
AB Stress is a way of life, and yeasts are no exception. The present communication summarizes studies on **tolerance in yeast** (essentially wild-type and **mutant** strains of *Saccharomyces cerevisiae*) to ethanol, **heat** and oxidative and free radical stresses. Tolerance to stress was measured by viable plate count and by fluorescence microscopy. In all cases and in all strains, a mild heat shock (25.degree. C to 37.degree. C or 42.degree. C for 30-60 min) induced tolerance to stress. However, the induced tolerance was transient, non-heritable and was not strongly correlated with stress protein synthesis. Stress tolerances were strongly growth phase dependent and in some cases also clearly strain dependent. Nevertheless, high monounsaturated fatty acid content (oleic acid), high sterol and high trehalose concns. appeared to have the best correlation with yeast stress tolerance.

L18 ANSWER 3 OF 17 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
AN 1999:433543 CAPLUS
DN 131:198814
TI **Stress tolerance** in doughs of *Saccharomyces cerevisiae* trehalase **mutants** derived from commercial baker's **yeast**
AU Shima, Jun; Hino, Akihiro; Yamada-Iyo, Chie; Suzuki, Yasuo; Nakajima, Ryouichi; Watanabe, Hajime; Mori, Katsumi; Takano, Hiroyuki
CS National Food Research Institute, Tsukuba, 305-8642, Japan
SO Appl. Environ. Microbiol. (1999), 65(7), 2841-2846
CODEN: AEMIDF; ISSN: 0099-2240
PB American Society for Microbiology
DT Journal
LA English
RE.CNT 33

RE
(1) App, H; J Biol Chem 1989, V264, P17583 CAPLUS
(2) Biswas, N; Biochim Biophys Acta 1997, V1335, P273 CAPLUS
(3) Bradford, M; Anal Biochem 1976, V72, P248 CAPLUS
(5) Brown, P; Methods Enzymol 1983, V101, P278 CAPLUS
(6) Chu, G; Science 1986, V234, P1582 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT
TI **Stress tolerance** in doughs of *Saccharomyces cerevisiae* trehalase **mutants** derived from commercial baker's **yeast**
IT Frozen foods
(frozen dough; **stress tolerance** in doughs of *Saccharomyces cerevisiae* trehalase **mutants** derived from com. baker's **yeast**)
IT Dough
(frozen; **stress tolerance** in doughs of *Saccharomyces cerevisiae* trehalase **mutants** derived from com. baker's **yeast**)
IT Bakers' **yeast**
Bread
Dough
Saccharomyces cerevisiae
(**stress tolerance** in doughs of *Saccharomyces*

cerevisiae trehalase mutants derived from com. baker's yeast)

IT Gene, microbial
 RL: BSU (Biological study, unclassified); FFD (Food or feed use); BIOL (Biological study); USES (Uses)
 (stress tolerance in doughs of Saccharomyces cerevisiae trehalase mutants derived from com. baker's yeast)

IT 124-38-9, Carbon dioxide, biological studies
 RL: MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
 (prodn. of; stress tolerance in doughs of Saccharomyces cerevisiae trehalase mutants derived from com. baker's yeast in relation to)

IT 99-20-7, Trehalose
 RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
 (stress tolerance in doughs of Saccharomyces cerevisiae trehalase mutants derived from com. baker's yeast)

IT 9025-52-9, Trehalase
 RL: BSU (Biological study, unclassified); FFD (Food or feed use); BIOL (Biological study); USES (Uses)
 (stress tolerance in doughs of Saccharomyces cerevisiae trehalase mutants derived from com. baker's yeast)

L18 ANSWER 4 OF 17 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2
 AN 1999:258621 CAPLUS
 DN 131:71216
 TI A Selaginella lepidophylla trehalose-6-phosphate synthase complements growth and stress-tolerance defects in a yeast tps1 mutant
 AU Zentella, Rodolfo; Mascorro-Gallardo, Jose O.; Van Dijck, Patrick; Folch-Mallol, Jorge; Bonini, Beatriz; Van Vaeck, Christophe; Gaxiola, Roberto; Covarrubias, Alejandra A.; Nieto-Sotelo, Jorge; Thevelein, Johan M.; Iturriaga, Gabriel
 CS Departamento de Biologia Molecular de Plantas, Instituto de Biotecnologia-Universidad Nacional Autonoma de Mexico, Cuernavaca Morelos, 62210, Mex.
 SO Plant Physiol. (1999), 119(4), 1473-1482
 CODEN: PLPHAY; ISSN: 0032-0889
 PB American Society of Plant Physiologists
 DT Journal
 LA English
 RE.CNT 43
 RE
 (1) Adams, R; Biochem Syst Ecol 1990, V18, P107 CAPLUS
 (2) Blazquez, M; Plant J 1998, V13, P685 CAPLUS
 (4) Cabib, E; J Biol Chem 1958, V231, P259 CAPLUS
 (5) Christianson, T; Gene 1992, V110, P119 CAPLUS
 (8) Colaco, C; Biotechnology 1992, V10, P1007 CAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT
 TI A Selaginella lepidophylla trehalose-6-phosphate synthase complements growth and stress-tolerance defects in a yeast tps1 mutant
 IT Complementation (genetic)
 Protein sequences
 Saccharomyces cerevisiae
 Selaginella lepidophylla
 Stress, microbial
 cDNA sequences
 (Selaginella lepidophylla trehalose-6-phosphate synthase complements growth and stress-tolerance defects in yeast tps1 mutant)

IT Gene, plant
 RL: PRP (Properties)
 (TPS1; Selaginella lepidophylla trehalose-6-phosphate synthase complements growth and **stress-tolerance** defects in **yeast tps1 mutant**)

IT 99-20-7, Trehalose
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (Selaginella lepidophylla trehalose-6-phosphate synthase complements growth and **stress-tolerance** defects in **yeast tps1 mutant**)

IT 9030-07-3, Trehalose 6-phosphate synthase
 RL: PRP (Properties)
 (Selaginella lepidophylla trehalose-6-phosphate synthase complements growth and **stress-tolerance** defects in **yeast tps1 mutant**)

IT 199877-30-0, Glucosyltransferase, uridine diphosphoglucose-glucose phosphate (Selaginella lepidophylla clone pIBT6 gene sl-tps/p)
 RL: PRP (Properties)
 (amino acid sequence; Selaginella lepidophylla trehalose-6-phosphate synthase complements growth and **stress-tolerance** defects in **yeast tps1 mutant**)

IT 199877-44-6, GenBank U96736
 RL: PRP (Properties)
 (nucleotide sequence; Selaginella lepidophylla trehalose-6-phosphate synthase complements growth and **stress-tolerance** defects in **yeast tps1 mutant**)

L18 ANSWER 5 OF 17 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3
 AN 1999:504068 CAPLUS
 DN 131:254851
 TI **Stress tolerance in a yeast lipid**
mutant: membrane lipids influence tolerance to heat and ethanol independently of heat shock proteins and trehalose

AU Swan, Tracey M.; Watson, Kenneth
 CS School of Biological Sciences, University of New England, Armidale, 2351, Australia
 SO Can. J. Microbiol. (1999), 45(6), 472-479
 CODEN: CJMIAZ; ISSN: 0008-4166
 PB National Research Council of Canada
 DT Journal
 LA English
 RE.CNT 60
 RE
 (1) Alexandre, H; Biotechnol Tech 1994, V8, P295 CAPLUS
 (2) Alexandre, H; FEMS Microbiol Lett 1994, V124, P17 CAPLUS
 (3) Attfield, P; FEBS Lett 1987, V225, P259 CAPLUS
 (5) Beaven, M; J Gen Microbiol 1982, V128, P1447 CAPLUS
 (6) Bowler, K; Temperature adaptation of biological membranes 1994, P185 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI **Stress tolerance in a yeast lipid**
mutant: membrane lipids influence tolerance to heat and ethanol independently of heat shock proteins and trehalose

IT Temperature effects, biological
 (heat, shock; **stress tolerance** in **yeast lipid mutant: membrane lipids influence tolerance to heat and ethanol independently of heat shock proteins and trehalose**)

IT Cell membrane
Mutation
 Saccharomyces cerevisiae
Stress, microbial
 (stress tolerance in yeast lipid)

mutant: membrane lipids influence tolerance to heat and ethanol independently of heat shock proteins and trehalose)

IT Heat-shock proteins
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(stress tolerance in yeast lipid
mutant: membrane lipids influence tolerance to heat and ethanol independently of heat shock proteins and trehalose)

IT Lipids, biological studies
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(stress tolerance in yeast lipid
mutant: membrane lipids influence tolerance to heat and ethanol independently of heat shock proteins and trehalose)

IT 64-17-5, Ethanol, biological studies 99-20-7, Trehalose
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(stress tolerance in yeast lipid
mutant: membrane lipids influence tolerance to heat and ethanol independently of heat shock proteins and trehalose)

L18 ANSWER 6 OF 17 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4
AN 1999:141606 CAPLUS
DN 131:99314
TI Cisplatin-modification of DNA repair and ionizing radiation lethality in yeast, *Saccharomyces cerevisiae*
AU Dolling, J.-A.; Boreham, D. R.; Brown, D. L.; Raaphorst, G. P.; Mitchel, R. E. J.
CS AECL, Radiation Biology and Health Physics Branch, Chalk River, ON, K0J 1J0, Can.
SO Mutat. Res. (1999), 433(2), 127-136
CODEN: MUREAV; ISSN: 0027-5107
PB Elsevier Science B.V.
DT Journal
LA English
RE.CNT 37
RE
(1) Alvarez, M; Br J Cancer 1978, V37, P68 CAPLUS
(3) Begg, A; Int J Radiation Oncology Biol Phys 1987, V13, P921 CAPLUS
(4) Boreham, D; Radiat Res 1991, V128, P19 CAPLUS
(5) Boreham, D; Radiat res 1990, V123, P203 CAPLUS
(7) Bruhn, S; Progress in Inorganic Chemistry: Bioinorganic Chemistry 1990, V38, P477 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB Cis-diamminedichloroplatinum II (cisplatin) is a DNA inter- and intrastrand crosslinking agent which can sensitize prokaryotic and eukaryotic cells to killing by ionizing radiation. The mechanism of radiosensitization is unknown but may involve cisplatin inhibition of repair of DNA damage caused by radiation. Repair proficient wild type and repair deficient (rad52, recombinational repair or rad3, excision repair) strains of the yeast *Saccharomyces cerevisiae* were used to det. whether defects in DNA repair mechanisms would modify the radiosensitizing effect of cisplatin. We report that cisplatin exposure could sensitize yeast cells with a competent recombinational repair mechanism (wild type or rad3), but could not sensitize cells defective in recombinational repair (rad52), indicating that the radiosensitizing effect of cisplatin was due to inhibition of DNA repair processes involving error free RAD52-dependent recombinational repair. The presence or absence of oxygen during irradiation.

did not alter this radiosensitization. Consistent with this result, cisplatin did not sensitize cells to mutation that results from lesion processing by an error prone DNA repair system. However, under certain circumstances, cisplatin exposure did not cause radiosensitization to killing by radiation in repair competent wild type cells. Within 2 h after a sublethal cisplatin treatment, wild type yeast cells became both thermally tolerant and radiation resistant. Cisplatin pretreatment also suppressed mutations caused by exposure to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), a response previously shown in wild type yeast cells following radiation pretreatment. Like radiation, the cisplatin-induced stress response did not confer radiation resistance or suppress MNNG mutations in a recombinational repair deficient mutant (rad52), although thermal tolerance was still induced. These results support the idea that cisplatin adducts in DNA interfere with RAD52-dependent recombinational repair and thereby sensitize cells to killing by radiation. However, the lesions can subsequently induce a general stress response, part of which is induction of RAD52-dependent error free recombinational repair. This **stress** response confers radiation resistance, thermal **tolerance**, and **mutation** resistance in **yeast**.

L18 ANSWER 7 OF 17 CAPLUS COPYRIGHT 2001 ACS

AN 1997:27037 CAPLUS

DN 126:115386

TI **Stress tolerant yeast mutants**

IN Klionsky, Daniel; Holzer, Helmut; Destruelle, Monika

PA University of California, USA

SO U.S., 17 pp.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5587290	A	19961224	US 1995-494714	19950626
	WO 9701626	A1	19970116	WO 1996-US10782	19960624
	W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN				
	AU 9663920	A1	19970130	AU 1996-63920	19960624
PRAI	US 1995-494714		19950626		
	WO 1996-US10782		19960624		
TI	Stress tolerant yeast mutants				
AB	The invention provides methods and compns. relating to stress tolerant yeast ; in particular, yeast mutants deficient in the expression of functional ATH1 gene product (Ath1p). Such yeast have enhanced tolerance to dehydration and freezing, are able to grow to a higher cell d. over a range of fermentable C source concns., and are able to produce and/or tolerate higher levels of ethanol and trehalose. Nucleic acids comprising ATH1 gene sequences are used in hybridization probes and PCR primers, in expression vectors, etc. The invention provides methods for producing a yeast mutant with improved survival ability under stress conditions which involve identifying mutations disrupting ATH1 expression using Ath1-specific reagents or ATH1 hybridization probes or primers.				
ST	stress tolerant yeast genetic mutation prodn				
IT	Genes (microbial)				
	RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)				

(ATH1; **stress-tolerant yeast mutants** deficient in expression of ATH1 gene product)

IT DNA sequences
Dehydration (physiological)
Freezing
Nucleic acid hybridization
PCR (polymerase chain reaction)
Protein sequences
Saccharomyces cerevisiae
(**stress-tolerant yeast mutants** deficient in expression of ATH1 gene product)

IT 186048-79-3P
RL: ARG (Analytical reagent use); BPR (Biological process); PUR (Purification or recovery); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(amino acid sequence; **stress-tolerant yeast mutants** deficient in expression of ATH1 gene product)

IT 186048-78-2P
RL: ARG (Analytical reagent use); BPR (Biological process); PUR (Purification or recovery); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(nucleotide sequence; **stress-tolerant yeast mutants** deficient in expression of ATH1 gene product)

IT 9025-52-9, Trehalase
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(**stress-tolerant yeast mutants** deficient in expression of ATH1 gene product)

IT 99-20-7, Trehalose
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(**stress-tolerant yeast mutants** deficient in expression of ATH1 gene product)

L18 ANSWER 8 OF 17 CAPLUS COPYRIGHT 2001 ACS
AN 1996:335076 CAPLUS
DN 125:77866
TI Two classes of plant cDNA clones differentially complement yeast calcineurin mutants and increase salt tolerance of wild-type yeast
AU Lippuner, Veronica; Cyert, Martha S.; Gasser, Charles S.
CS Section Mol. Cell. Biol., Univ. California, Davis, CA, 95616, USA
SO J. Biol. Chem. (1996), 271(22), 12859-12866
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
IT Plant **stress**
(salinity, Arabidopsis thaliana genes STO and STZ differentially complement **yeast calcineurin mutants** and increase salt **tolerance** of wild-type yeast)

L18 ANSWER 9 OF 17 CAPLUS COPYRIGHT 2001 ACS
AN 1996:511929 CAPLUS
DN 125:190460
TI Regulation of intracellular osmotic pressure and some factors that influence the promotion of glycerol synthesis in a respiration-deficient **mutant of the salt-tolerant yeast**
Zygosaccharomyces rouxii during salt **stress**
AU Ohshiro, Kyouichi; Yagi, Tadashi
CS Faculty Science, Osaka City University, Osaka, 558, Japan
SO J. Gen. Appl. Microbiol. (1996), 42(3), 201-212
CODEN: JGAMA9; ISSN: 0022-1260
DT Journal
LA English
TI Regulation of intracellular osmotic pressure and some factors that influence the promotion of glycerol synthesis in a respiration-deficient **mutant of the salt-tolerant yeast**

Zygosaccharomyces rouxii during salt stress

AB The accumulation of glycerol and inorg. ions were amd. in a respiration-deficient (RD) mutant isolated from the salt-tolerant yeast Zygosaccharomyces rouxii for 3 h after salt stress due to 1 M NaCl. After the start of salt stress, intracellular levels of glycerol continued to increase for up to 3 h, while the levels of Na⁺ and Cl⁻ ions in cells reached max. values within 1 h and then decreased gradually. Increases in intracellular concns. of solutes resulted in an osmotic pressure that was almost equiv. to the external osmotic pressure within 2 h after salt stress. The RD strain had the same ability to tolerate salt as the wild-type strain. Therefore, we used the RD strain to examine the mechanism in the glycolytic pathway that is responsible for the promotion of glycerol synthesis that is induced by NaCl. When exposed to medium with 1 M NaCl, RD cells diverted about one-sixteenth of the amt. of ethanol that was produced in the medium without NaCl to the prodn. of glycerol. This result suggests the presence of factors that mediate a change from the normal metab. of glucose to the promotion of glycerol synthesis in response to external NaCl. The specific activities of glycerol-3-phosphate dehydrogenase (GPDH) in exts. of cells grown with and without 1 M NaCl were very low in reaction mixts. with NADH or NADPH, although the cellular activity of alc. dehydrogenase (ADH) was high and was repressed by external NaCl. This result indicates that the pathway involving GPDH makes only a small contribution to the synthesis of glycerol and that an alternative pathway functions for the synthesis in Z. rouxii. The addn. of sodium sulfite, which binds to acetaldehyde, and of glycidol, an inhibitor of triose phosphate isomerase (TPI), to the medium promoted the synthesis of glycerol in RD strain. These results suggest the possibility that the extra NADH resulted from the binding of sulfite to acetaldehyde, or the inhibition of ADH and/or TPI under the NaCl-stressed condition lead to the promotion of glycerol synthesis by Z. rouxii.

L18 ANSWER 10 OF 17 CAPLUS COPYRIGHT 2001 ACS

AN 1997:336759 CAPLUS

DN 127:1246

TI Analysis of multiple classes of soybean heat shock genes and proteins

AU Nagao, Ron T.; Lee, Yuh-Ru Julie; Lafayette, Peter R.; Goekjian, Virginia H.; O'grady, Kevin; Key, Joe L.

CS Department of Botany, University of Georgia, Athens, GA, 30602, USA

SO Phys. Stresses Plants: Genes Their Prod. Tolerance, Proc. Workshop (1996),

Meeting Date 1995, 3-20. Editor(s): Grillo, Stefania; Leone, Antonella.

Publisher: Springer, Berlin, Germany.

CODEN: 64JRAU

DT Conference; General Review

LA English

AB A review with 78 refs. The influence of high temp. stress (heat shock or HS) and other environmental stress agents on gene expression of soybean seedlings has been extensively studied. The sequence anal. of HS genes has revealed a high degree of conservation among individual members of several heat shock protein (HSP) families and different classes within a family, but some interesting differences have been noted. These studies have also revealed complex patterns of regulation of expression of the HS genes and accumulation of the HSPs. Based primarily upon the deduced amino acid sequence of the HSPs, immunol. cross-reactivity, and intracellular localization, the complex group of low mol. wt. (LMW) HSP genes have been organized into multiple classes. In soybean several cDNA and genomic clones encoding 20 to 24 kDa LMW HSPs have been isolated

which represent new classes of the LMW HSP gene super family based on nucleotide/amino acid sequence and cell fractionation analyses. The mRNAs

transcribed from these genes are of lower abundance than those for the 15 to 18 kDa Class I and II proteins, and these genes occur as small multigene (i.e. three to four) classes or subfamilies. The mRNAs of three of these classes of LMW HSP genes are translated on ER-bound ribosomes and possess hydrophobic leader sequences. The presence of a consensus ER retention sequence on two of these proteins indicates that they probably reside within the ER. The third protein lacks the consensus ER retention signal and presumably is translocated to an as yet unidentified location. The mRNA representing a fourth LMW gene class is translated on unbound cytoplasmic ribosomes, and the predicted protein has a N-terminal sequence with properties similar to that of some proteins which are translocated into mitochondria. Early studies with soybean seedlings indicated that some 22 to 24 kDa HSPs are localized in mitochondria. Differential induction by amino analog treatment indicates that genes assigned to the same class based on amino acid similarity and localization can be regulated differently. The possible role of the multiple classes on LMW 15 to 24 kDa HSPs in protein protection from denaturation at high temp. (i.e. a chaperone function), based on studies from other labs. is noted and some of these results will be summarized. One aspect of the physiol./biochem. role(s) of HSPs in cellular function was studied by my lab., emphasizing the phenomenon of acquired thermotolerance. A soybean HSP101 gene was isolated and sequenced. This soybean gene is homologous to the yeast HSP104 gene and was used to complement a yeast HSP104 deletion mutant in the acquisition of thermotolerance. Results of these expts. demonstrate that the soybean gene can partially restore heat tolerance in the yeast deletion mutant indicating that soybean HSP101 is functionally similar to yeast HSP104. The HSP101 gene family is again one of several groups or gene families for high mol. wt. HSPs (e.g. HSP70s, HSP80s, HSP60s and HSP90/92s). Studies on these other HSPs/HSP genes will be reviewed along with the presentation of some of the newer results from our lab. Results from a no. of studies in many labs. support the view that these HSPs function as chaperones in multiple types of protein-protein.

L18 ANSWER 11 OF 17 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 5
 AN 1995:978238 CAPLUS
 DN 124:111837
 TI Trehalase activity and trehalose content in a **freeze-tolerant yeast**, *Torulaspora delbrueckii*, and its **freeze-sensitive mutant**
 AU Yokoigawa, Kumio; Murakami, Yoko; Kawai, Hiroyasu
 CS Dep. Food Science Nutrition, Nara Women's Univ., Nara, 630, Japan
 SO Biosci., Biotechnol., Biochem. (1995), 59(11), 2143-5
 CODEN: BBBIEJ; ISSN: 0916-8451
 DT Journal
 LA English
 TI Trehalase activity and trehalose content in a **freeze-tolerant yeast**, *Torulaspora delbrueckii*, and its **freeze-sensitive mutant**
 IT Yeast
 (freeze-sensitive and **freeze-tolerant**; trehalase activity and trehalose content in **freeze-tolerant yeast**, *Torulaspora delbrueckii*, and **freeze-sensitive mutant**)
 IT *Torulaspora delbrueckii*
 (trehalase activity and trehalose content in **freeze-tolerant yeast**, *Torulaspora delbrueckii*, and **freeze-sensitive mutant**)
 IT 99-20-7, Trehalose
 RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)

(trehalase activity and trehalose content in **freeze-tolerant yeast**, *Torulaspora delbrueckii*, and **freeze-sensitive mutant**)

IT 9025-52-9, Trehalase
 RL: BOC (Biological occurrence); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
 (trehalase activity and trehalose content in **freeze-tolerant yeast**, *Torulaspora delbrueckii*, and **freeze-sensitive mutant**)

L18 ANSWER 12 OF 17 CAPLUS COPYRIGHT 2001 ACS
 AN 1996:204707 CAPLUS
 DN 125:5185
 TI Lipid composition of a **freeze-tolerant yeast**, *Torulaspora delbrueckii*, and its **freeze-sensitive mutant**
 AU Murakami, Y.; Yokoigawa, K.; Kawai, H.
 CS Department of Food Science and Nutrition, Nara Women's University, Nara, 630, Japan
 SO Appl. Microbiol. Biotechnol. (1995), 44(1-2), 167-71
 CODEN: AMBIDG; ISSN: 0175-7598
 DT Journal
 LA English
 TI Lipid composition of a **freeze-tolerant yeast**, *Torulaspora delbrueckii*, and its **freeze-sensitive mutant**
 AB The lipid compns. of a **freeze-tolerant** strain of **yeast** *Torulaspora delbrueckii* D2-4 and its **freeze-sensitive mutant** 60B3 were analyzed to clarify the relationship between the lipid compn. and freeze tolerance of yeast. The total lipid content of D2-4 was similar to that of 60B3, whereas the content of phospholipids and neutral lipids was different from those of 60B3. The molar ratio of sterol to phospholipid in D2-4 was 60% of that in 60B3. The anal. of lipid components indicated that D2-4 contained larger amts. of phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol, but smaller amts. of triglyceride as compared to 60B3. Thus, the plasma membrane of freeze-tolerant strain D2-4 may have
 a higher fluidity than that of freeze-sensitive strain 60B3.

IT **Freezing**
Torulaspora delbrueckii
 (lipid compn. of **freeze-tolerant yeast**, *Torulaspora delbrueckii*, and its **freeze-sensitive mutant**)

IT Cardiolipins
 Fatty acids, biological studies
 Glycerides, biological studies
 Lipids, biological studies
 Phosphatidic acids
 Phosphatidylcholines, biological studies
 Phosphatidylethanolamines
 Phosphatidylinositols
 Phosphatidylserines
 Phospholipids, biological studies
 RL: BOC (Biological occurrence); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
 (lipid compn. of **freeze-tolerant yeast**, *Torulaspora delbrueckii*, and its **freeze-sensitive mutant**)

IT Steroids, biological studies
 RL: BOC (Biological occurrence); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
 (hydroxy, lipid compn. of **freeze-tolerant yeast**, *Torulaspora delbrueckii*, and its **freeze-sensitive mutant**)

L18 ANSWER 13 OF 17 CAPLUS COPYRIGHT 2001 ACS
 AN 1995:459144 CAPLUS
 DN 122:234891
 TI Breeding of freeze-tolerant yeasts using improvements of the pathway of trehalose metabolites
 AU Hino, Akihiro
 CS Biotechnol. Div., Agric., For. Fish. Res. Counc. Secur., Tokyo, 100, Japan
 SO Baiosaiensu to Indasutori (1995), 53(1), 29-31
 CODEN: BIDSE6; ISSN: 0914-8981
 DT Journal; General Review
 LA Japanese
 AB A review with 12 refs. on roles of trehalose in **freeze-tolerant yeasts**, characteristics of **mutants** with constitutive expression of GGS1 gene and neg.-NTH1 gene, and correlation of trehalose contents with freeze-tolerance.

L18 ANSWER 14 OF 17 CAPLUS COPYRIGHT 2001 ACS
 AN 1994:158599 CAPLUS
 DN 120:158599
 TI Induction of **freeze-sensitive mutants** from a **freeze-tolerant yeast**, *Torulaspora delbrueckii*
 AU Murakami, Yoko; Hahn, Young Sook; Yokoigawa, Kumio; Endo, Kinji; Kawai, Hiroyasu
 CS Dep. Food Sci. Nutr., Nara Women's Univ., Nara, 630, Japan
 SO Biosci., Biotechnol., Biochem. (1994), 58(1), 206-7
 CODEN: BBBIEJ; ISSN: 0916-8451
 DT Journal
 LA English
 TI Induction of **freeze-sensitive mutants** from a **freeze-tolerant yeast**, *Torulaspora delbrueckii*
 AB **Freeze-sensitive** strains of yeast were induced from a **freeze-tolerant yeast** *Torulaspora delbrueckii* by incubation with ethylmethane sulfonate as a **mutagen**. A max. ratio of mutation was attained by the incubation at 30 .degree.C for 75 min. Some 150 strains of freeze-sensitive yeasts were selected by plating-culture for the first screening. The freeze-tolerance ratio of each strain was examd. based on the fermm. activity before and after freezing in liq. medium and dough. Strain 60B3 showed the highest freeze sensitivity, in a pre-fermented frozen dough (pre-fermented at 30 .degree.C for 2 h, and frozen at -20 .degree.C for 7 days) among eight strains finally selected.

L18 ANSWER 15 OF 17 CAPLUS COPYRIGHT 2001 ACS
 AN 1990:627913 CAPLUS
 DN 113:227913
 TI Physical and biochemical properties of **freeze-tolerant mutants** of a **yeast** *Saccharomyces cerevisiae*
 AU Matsutani, Keiko; Fukuda, Yasuki; Murata, Kousaku; Kimura, Akira; Nakamura, Ichiro; Yajima, Norio
 CS Chukyo Community Coll., Mizunami, 509-61, Japan
 SO J. Ferment. Bioeng. (1990), 70(4), 275-6
 CODEN: JFBIEX; ISSN: 0922-338X
 DT Journal
 LA English
 TI Physical and biochemical properties of **freeze-tolerant mutants** of a **yeast** *Saccharomyces cerevisiae*

L18 ANSWER 16 OF 17 MEDLINE
 AN 85157424 MEDLINE
 DN 85157424 PubMed ID: 3980438
 TI Glycerol metabolism and osmoregulation in the salt-tolerant yeast *Debaryomyces hansenii*.
 AU Adler L; Blomberg A; Nilsson A

SO JOURNAL OF BACTERIOLOGY, (1985 Apr) 162 (1) 300-6
 Journal code: HH 2985120R. ISSN: 0021-9193.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 198505

ED Entered STN: 19900320
 Last Updated on STN: 19980206
 Entered Medline: 19850509

AB A glycerol-nonutilizing **mutant** of the salt-tolerant **yeast** *Debaryomyces hansenii* was isolated. When subjected to salt **stress** the **mutant** produced glycerol, and the internal level of glycerol increased linearly in proportion to increases of external salinity as in the wild-type strain. However, at increased salinity the mutant showed a more pronounced decrease of growth rate and growth yield and lost more glycerol to the surrounding medium than did the wild type. Uptake experiments showed glycerol to be accumulated against a strong concentration gradient, and both strains displayed similar kinetic parameters for the uptake of glycerol. An examination of enzyme activities of the glycerol metabolism revealed that the apparent Km of the sn-glycerol 3-phosphate dehydrogenase (EC 1.1.99.5) was increased 330-fold for sn-glycerol 3-phosphate in the mutant. Based on the findings, a scheme for the pathways of glycerol metabolism is suggested.

L18 ANSWER 17 OF 17 CAPLUS COPYRIGHT 2001 ACS

AN 1983:451841 CAPLUS

DN 99:51841

TI Use of N-nitrosomethylurea for producing quick-growing **heat-tolerant mutant** varieties of **yeasts** under continuous cultivation

AU Amirbaeva, M. I.; Tulemisova, K. A.; Dubitskaya, S. D.

CS USSR

SO Deposited Doc. (1982), VINITI 3337-82, 10 pp. Avail.: VINITI

DT Report

LA Russian

TI Use of N-nitrosomethylurea for producing quick-growing **heat-tolerant mutant** varieties of **yeasts** under continuous cultivation